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The Effect of Ovarian Hormones on Antioxidant Enzyme Activities in the Brain of Male Rats

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Summary

The brain is widely responsive to gonadal hormones. The functional significance of ovarian hormones in the brain is evident from biochemical studies indicating that estradiol or progesterone treatment of testectomized rats produces changes of antioxidant enzyme activities. The effect of estradiol benzoate (EB) and progesterone (P) in the control of antioxidant (AO) enzyme activities was studied in the brain of adult male Wistar rats. The activities of catalase (CAT), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) and glutathione reductase (GR) were measured in appropriate subcellular fractions, prepared from brains of animals belonging to various experimental groups. These groups were designed with the intention to follow changes in enzyme activities 2 h or 24 h after systemic administration of 5 μg EB or 2 mg P to testectomized (TX) animals. The obtained results show that both EB and P increase CAT activity, whereas EB decreases GSH-Px, GST and GR activities. These findings clearly show the modulatory role of EB and P in the control of enzymes responsible for the protection of rat nerve cells against oxidative damage caused by free oxygen radicals.

Key words
Brain • Antioxidant enzymes • Estradiol • Progesterone • Rats

Introduction

Oxyradicals (reactive oxygen species) are generated continuously in neurons during normal metabolism and neuronal activity. Whether damage occurs or further develops depends on the balance between the generation of oxyradicals and the activity of cellular antioxidant defenses. When the generation of oxyradicals exceeds the capacity of intracellular defense and repair mechanisms or the function of anti-oxidant defenses is impaired, the cell dies (Allen and Tresini 2000).

Oxidative stress is the cytotoxic consequence of oxyradical and oxidant formation and the reaction with cellular constituents. The free radical $O_2^{\cdot-}$ is generated by multiple enzymatic and non-enzymatic pathways and is often at the start of the oxidative stress cascade (Allen 1991).

Several enzymes are important in the antioxidant defense system (AOS), because they metabolize either free radicals or reactive oxygen intermediates to non-radical products. These enzymes include a family of glutathione-dependent enzymes known as glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST)

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and glutathione reductase (GR), (Chaudiere and Ferrari-Iiou 1999). On the other hand, sex steroid hormones regulate the activities of a number of enzymes of the antioxidant defense system (Huh *et al.* 1994, Pajović *et al.* 1999, Azevedo *et al.* 2001). Thus, Azevedo *et al.* (2001) investigated the regulation of AO enzyme activities in male and female rat macrophages by sex steroids. They concluded that the differences in the SOD and CAT activities might partially explain some of the differences in immune functions reported for males and females. Furthermore, the values of estrogen, a potent regulator of CAT in macrophages, may vary considerably during the menstrual cycle. Barbacanne *et al.* (1999) concluded that estradiol increases endothelium-derived relaxing factor (EDRF) activity in the rat aorta in the absence of changes in endothelial NO synthase gene expression. The decreased endothelium-derived generation of O_2^- in response to estrogens could account for enhanced EDRF-NO bioactivity and decreased peroxynitrite release. All of these effects could contribute to the vascular protective properties of estrogens.

Tiidus *et al.* (1998) showed that the estrogen administration had little effect on post-exercise tissue glutathione status, SOD and GSH-Px activities and vitamin E levels. Estrogen administration induced significant reductions of vitamin C concentrations in muscles, the liver and heart following exercise as well as in non-exercised male rats. Thus, estrogen administration generally did not appear to influence post-exercise tissue indices of oxidative stress or antioxidant status and may have contributed to a decline in overall antioxidant protection by inducing losses in tissue vitamin C.

Kume-Kick and Rice (1998) examined estrogen-dependent modulation of rat brain ascorbate levels and ischemia-induced ascorbate loss. Progesterone had little effect in any region. These data indicate that the ascorbate content and redox balance in female brain are influenced postpubertally by estrogens in a region-selective manner. In our previous work, the activity of antioxidant defense enzymes in the brain has been shown to vary during the estrous cycle, to be altered following gonadectomy and to differ between the sexes, suggesting involvement of gonadal hormones in the control of processes which protect cells and tissues against oxidative damage (Pajović *et al.* 1996, Saičić *et al.* 1998).

In the present work, we studied the influence of progesterone and estradiol 17-β benzoate on the antioxidant enzyme activities: catalase (CAT, EC 1.11.1.6.), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione-S-transferase (GST, EC 2.5.1.18) and glutathione reductase (GR EC 1.6.4.2.) in the brain of male rats.

Methods

Bilaterally testectomized male Wistar rats, aged 3.5 months, were used in our experiments. They were kept in large open-colony cages under controlled conditions of illumination (lights on 0:05-17:00 h) and temperature (23±2 ^oC), and were allowed free access to water and food. All chemicals were from Sigma (St. Louis, Mo. U.S.A.).

Two hours or 24 h before sacrifice, a single injection of 2 mg P (progesterone, Sigma), or 5 μg EB (3-estradiol-3-benzoate, Sigma), suspended in 0.1 ml olive oil, was given subcutaneously (sc) to testectomized animals. Controls received olive oil alone.

Animals were killed by decapitation with a guillotine (Harvard Apparatus) and fresh whole brains were dissected out and subjected to homogenization. Homogenization was performed in a Janke and Kunkel (Staufen, Germany) Ka-Werk Ultra-Turrax homogenizer at 0-4 ^oC using 0.25 mol/l sucrose, 1 mmol/l EDTA and 0.05 mol/l Tris-HCl solution, pH 7.4 (Rossi *et al.* 1983, De Waziers and Albrecht 1987). The homogenates were centrifuged (90 min, 85000 x *g,* 4 ^oC) and the supernatant was used for antioxidant enzyme activity assays and total protein determination.

CAT activity was assayed as suggested by Beutler (1982) and expressed in μmol H_2O_2/min/mg protein. The method is based on the rate of H_2O_2 degradation by the action of CAT contained in the samples, monitored spectrophotometrically at 230 nm in 5 mmol/l EDTA, Tris-HCl solution, pH 8.0. The activity of GSH-Px was measured using t-butyl hydroperoxide as a substrate (Paglia and Valentine 1967) as modified by Tamura *et al.* (1982). The activity was expressed as nmol of NADPH oxidized/min/mg protein. For determination of GST activity, 1-chloro-2,4-dinitro benzene (CDNB) was used as a substrate (Habig *et al.* 1974) and the activity was expressed as nmol GSH used/min/mg protein. GR activity was assayed as suggested by Glatzle *et al.* (1974) and expressed as nmol NADPH

oxidized/min/mg protein. Protein concentrations were determined by the method of Lowry *et al.* (1951).

The results were analyzed by Student's t-test and by ANOVA. Differences between means were considered significant at the 5 % level.

Results

Effects of P and EB on CAT activity

The post-castration activity of brain CAT appeared to be steady after treatment with 2 mg P, given to TX animals 2 h before sacrifice (Fig. 1). The values for control TX and TX+P treated animals after 2 h were 9.7±0.7 and 7.7±1.2 U/mg protein ($F_{(1,4)}$ = 2.66, p>0.05), respectively. However, 24 h after treatment with P the activity of CAT was significantly increased (8.7±0.7 and 10.9±0.3 U/mg; $F_{(1,4)}$=7.65, p<0.05). The values for control TX and TX+EB treated animals were 9.7±0.7 and 12.0±1.4 U/mg protein ($F_{(1,4)}$ = 2.87; p>0.05) after 2 h, and 8.7±0.7 and 8.5±0.8 U/mg protein ($F_{(1,4)}$ = 0.07; p>0.05) after 24 h, respectively.

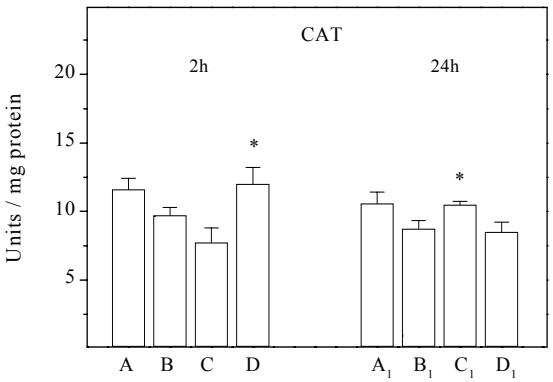

Fig. 1. *CAT activity in brain homogenates of long-term testectomized rats treated 2 h and 24 h prior to sample preparation; A: non-treated controls (n=4); B: controls treated with olive oil (n=4); C: treated with 2 mg P (n=4); D: treated with 5 μg EB (n=4). Data are means ± S.E.M.; B : D - * p<0.05; B_1 : C_1 - * p<0.05*

Effects of P and EB on GSH-Px activity

The GSH-Px activity in brain homogenates of TX animals were not affected by the P treatment (Fig. 2). The corresponding values for control TX and TX+P treated animals were 40.0±2.3 and 39.5±2.6 U/mg protein at 2 h (F=0.02; p>0.05) and 40.3±2.6 and 42.9±2.9 U/mg protein at 24 h ($F_{(1,4)}$=0.58; p>0.05). The GSH-Px activity was steady 24 h after administration of EB (control TX 40.3±2.6 and TX+EB 38.7±3.8 U/mg protein; ($F_{(1,4)}$ = 0.17, p>0.05) (Fig. 2). On the other hand, a marked decrease of GSH-Px activity in TX rats was obtained with 5 μg EB at 2 h after treatment (control TX 40.0±2.3 and TX+EB 33.0±3.5 U/mg protein; $t_{(6)}$=1.94, p<0.05) (Fig. 2).

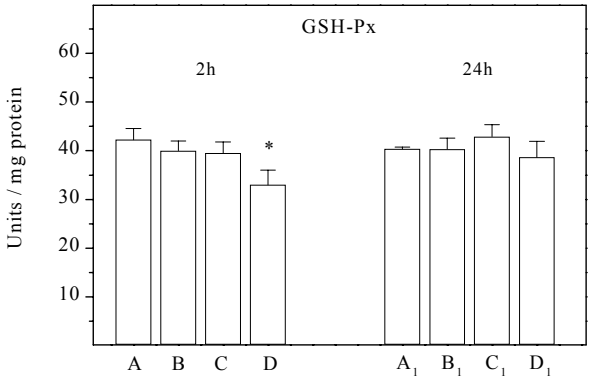

Fig. 2. *GSH-Px activity in brain homogenates of long-term testectomized rats treated 2 h and 24 h prior to sample preparation; A: non-treated controls (n=4); B: controls treated with olive oil (n=4); C: treated with 2 mg P (n=4); D: treated with 5 μg EB (n=4). Data are means ± S.E.M.; B : D - * p<0.05*

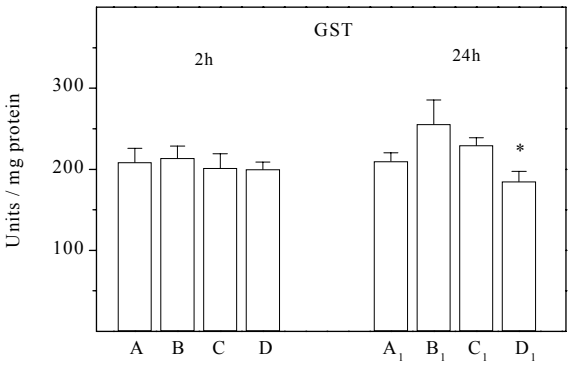

Fig. 3. *GST activity in brain homogenates of long-term testectomized rats treated 2 h and 24 h prior to sample preparation; A: non-treated controls (n=4); B: controls treated with olive oil (n=4); C: treated with 2 mg P (n=4); D: treated with 5 μg EB (n=4). Data are means ± S.E.M.; B_1 : D_1 - * p<0.05*

Effects of P and EB on GST activity

In TX rats, the activity of GST was not influenced by 2 mg of P given to TX animals either 2 h or 24 h before sacrifice; at 2 h: control TX 213.5±17.3 and

TX+P 201.4±20.7,($F_{(1,4)}$=0.27, p>0.05); at 24 h: control TX 255.7±34.3 and TX+P 229.4±11.1, ($F_{(1,4)}$=0.71, p>0.05) (Fig. 3). The activity of brain GST was also not affected by EB 2 h after treatment; the respective values for control TX and TX+EB treated animals were 213.5±17.3 and 199.9±13.6 U/mg protein ($F_{(1,4)}$=0.61, p>0.05). However, 24 h after treatment the activity of GST was significantly decreased by EB (control TX 255.7±34.3 and TX+EB 184.8±14.7 U/mg protein) ($t_{(6)}$= 2.19, p<0.05) (Fig. 3).

Effects of P and EB on GR activity

Systemic administration of 5 μg EB to TX rats evoked a significant decrease of GR activity in the brain 24 h later (control TX 39.7±1.3 and TX+EB 35.4±1.2 U/mg protein) ($F_{(1,4)}$=8.15, p<0.05) (Fig. 4). GR was not significantly decreased 2 h after administration EB (control TX 43.3±1.7 and TX+EB 42.9±2.1 U/mg protein) ($F_{(1,4)}$=0.03, p>0.05) (Fig. 4). In TX rats, the activity of GR was not influenced by 2 mg of P given to TX animals either 2 h or 24 h before sacrifice; at 2 h: control TX 43.3±1.7 and TX+P 42.5±1.72, ($F_{(1,4)}$=0.15, p>0.05); at 24 h: control TX 39.7±1.3 and TX±P 39.1±1.7, ($F_{(1,4)}$=0.09, p>0.05) (Fig. 4).

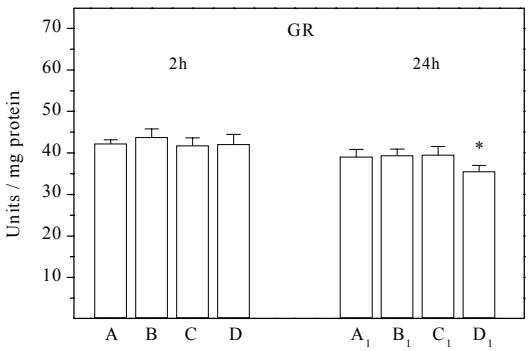

Fig. 4. *GR activity in brain homogenates of long-term testectomized rats treated 2 h and 24 h prior to sample preparation; A: non-treated controls (n=4); B: controls treated with olive oil (n=4); C: treated with 2 mg P (n=4); D: treated with 5 μg EB (n=4). Data are means ± S.E.M.; B_1 : D_1 - * p<0.05*

Discussion

The inherent biochemical and physiological characteristics of the brain, including high lipid concentrations and energy requirements, make it particularly susceptible to free radical-mediated insult. The brain, like many other tissues, has a range of antioxidant defenses, which help to maintain a balanced redox status. Male rats have a higher extent of lipid peroxidation than females (Huh *et al.* 1994). On the basis of these considerations, we investigated the role of ovarian hormones in the protection against oxidative stress in the brain. We compared the activities of antioxidant enzymes: CAT, GSH-Px, GST and GR in the brain of male rats and examined the effect of sex steroid hormone replacement in the nervous tissue of testectomized male rats. This study has shown that the ovarian hormones induced CAT activity, whereas only EB suppressed GSH-Px, GST and GR activities in the brain of male rats. The activity of CAT and GSH-Px was changed after 2 h, while the activity of GR and GST did not change until 24 h after estrogen administration. On the other hand, progesterone induced the activity of CAT only 24 h after treatment. In the light of these data, it may be concluded that the differences between ovarian hormones in the effect on the activity of studied antioxidant enzymes result from the different pathways of their action. Furthermore, the modulatory effects of the hormone and the other microenvironmental factors on the enzyme catalytic rates are very specific and significant for each antioxidant enzyme (Nagy and Floyd 1991). In accordance with this, the activity of GST markedly enhanced 24 h after the administration of olive oil in contrast with other enzymes. The results suggest that some compounds from olive oil may induce GST which has potent antilipoperoxidative and detoxifying effects.

Nevertheless, the involvement of endogenous antioxidant enzymes in the protective effect against oxidative stress in nervous tissue is controlled by ovarian hormones. A number of results have implied that there exists a correlation between the antioxidant status and hormonal levels (Inal *et al.* 1997, Rapoport *et al.*1998, Cardozo-Pelaez *et al.* 2000). Thus, Inal *et al.* (1997) investigated the effects of postmenopausal hormone replacement and alpha-tocopherol on the lipid profiles and antioxidant status. They concluded that the hormone replacement and vitamin E combined therapy is effective in prevention of cardiovascular diseases. In order to investigate the role of hormonal regulation of the antioxidative defense system in bovine corpora lutea, Rapoport *et al.* (1998) determined the levels of some antioxidants and antioxidative enzymes at different

developmental stages of corpora lutea. The correlation between these levels and progesterone levels indicates that antioxidative mechanisms are activated to cope with steroidogenesis-dependent oxyradical formation in the bovine corpus luteum.

Oxidative damage of DNA usually involves damage to single bases. It is estimated that at least 35 different base modifications are formed in reactions with reactive oxygen species (ROS) (McBride *et al.* 1991). The array of base damage differs depending on the ROS that damages DNA (Aruoma *et al.* 1989). Thymidine glycol and 8-hydroxy-deoxyguanosine have received the most attention among the lesions discovered. Because they are not intermediates in normal nucleotide metabolism, they may therefore be used as biomarkers for oxidative DNA damage (Simic *et al.* 1994). Cardozo-Pelaez *et al.* (2000) have shown correlation between DNA damage, repair and antioxidative defense system in some brain regions. Oxidative stress affects some brain regions more than others, as demonstrated by regional differences in steady-state 8-hydroxy-2'-deoxyguanosine (oxo(8)dG) levels in the mouse brain. Although each brain region exhibits significant differences in antioxidant defenses, the capacity to excise the oxidized base from DNA seems to be the major determinant of the steady-state levels of oxo (8)dG in each brain region.

In conclusion, understanding the role of oxyradicals and identifying the critical species in different neurodegenerative diseases, will hopefully lead to the development of selective therapeutic agents and to a better understanding of basic processes underlying normal and pathological neuronal functions.

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